TOPIC 01-2 – Myocardial infarction

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0300

The envelope protein of a human endogenous retrovirus exerts inflammatory activity on a blood brain barrier model through TLR4 receptor

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The MSRV (Multiple Sclerosis Associated Retro Virus) belongs to human endogenous retrovirus HERV-W family. An envelope protein originating from the MSRV was found in most patients with multiple sclerosis (MS). This protein (ENV-MS) has pro-inflammatory properties on several immune cells and could therefore play a role in the MS pathogenesis by promoting the leukocyte diapedesis observed in the central nervous system of patients. Our study aims to analyze the effects of ENV-MS on the blood-brain barrier (BBB) at a molecular and functional level.

So far we have demonstrated that a recombinant MSRV envelope was able to strongly stimulate several inflammatory parameters on a human BBB model. Indeed, ENV-MS induced overexpression of ICAM-1 in a dose-dependent manner and a strong dose-dependent production of the inflammatory cytokines IL-6 and IL-8. Furthermore, we have shown that ENV-MS was recognized *via* the TLR4 receptor (Toll Like Receptor 4), a pattern recognition receptor present on the membrane of endothelial cells. At a functional level, we have also shown that the treatment with ENV-MS significantly stimulated the adhesion of activated immune cells to a monolayer of endothelial cells.

These findings support the hypothesis that MSRV could be involved in the pathogenesis of MS disease or at least in maintenance of inflammatory conditions thus fueling the auto-immune disorder.

Ongoing work, at functional level, is to measure the BBB model permeability after treatment with ENV-MS using transwell assays. These experiments should allow us to demonstrate that ENV-MS induces an increase of transmigration of immune cells across the monolayer.

Finally, we are currently developing animal models. These animals are transgenic mice expressing MSRV envelope with an inducible expression system. This model should allow us to study the proinflammatory effects of MSRV in a model closer to physiopathological conditions.

0131

A new form of LAD-III (Leukocyte Adhesion Deficiency): The absence of kindlin-3 reduces leukocytes adhesion by mechanisms dependent and independent of integrins activation

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Introduction: In the present study we describe the case of a young patient from gipsy origin. He had to be hospitalized because of an important bleeding disorder mainly observable on skin and mucosa.

Results: Platelet functional study directed the diagnosis towards a Glanzmann thrombasthenia. Surprisingly, genetic analysis highlighted that the patient was heterozygous for the French gipsy mutation (1544+1G>A transition in the intron 15 of the *ITGA2B* gene) incompatible with the observed phenotype. Even though, the αIIbβ3 integrin surface expression appeared to be normal, the patient's platelets were unable to unmask the fibrinogen binding site in response to various agonists. Moreover, the patient presented a moderate immune deficiency indicating a more diffuse defect similar to a type III Leukocyte Adhesion Deficiency variant (LADIII variant). This pathology was recently associated to mutations in the *FERMT3* gene that is responsible for the abnormal expression of the kindlin-3 protein. It is strongly involved in the integrin activation process. Here we report the first French case of LAD-III caused by a newly discovered mutation in the FERMT3 gene. The kindlin-3 deficient leukocytes are able to easily form initial bounds in response to a chemotactic agent or to a phorbol ester. However, the patient's neutrophils and lymphocytes displayed a reduced ability to strengthen preformed bounds compared to leukocytes isolated from his parents and healthy volunteers. Furthermore, the integrin dependent and independent spreading ability of the patient's T lymphocytes was markedly reduced.

Conclusion: The effects of a kindlin-3 deficiency on integrin function are mainly dependent on the cell type studied and on the stimulus causing their activation. Our results also highlight the possible implication of kindlin-3 in integrins independent mechanisms.

0193

The chemokine decoy receptor D6 prevents excessive inflammation and adverse ventricular remodelling after myocardial infarction

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Infiltration of leukocytes in ischemic areas is a hallmark of myocardial infarction (MI), and overwhelming inflammation promotes adverse ventricular remodeling. Recruitment of leukocytes following MI partially depends on inflammatory CC-chemokines. We hypothesized that the chemokine decoy receptor D6, that specifically recognizes and scavenges inflammatory CC-chemokines, might prevent excessive inflammation and adverse remodelling after MI. In a murine model of MI, D6 expression in the ischemic left ventricle raised by 250% at 5 and 7 days after the onset of ischemia ($p<0.001$). Protein levels of CCL2 and CCL3, two D6 targets, were increased in the ischemic myocardium, peaking at day 3 ($p<0.001$ vs baseline), and progressively returning to basal levels between days 5 and 7. Infiltration of CD45+ leukocytes in the ischemic myocardium of D6-/- mice was increased by 180% ($p<0.05$) and 200% ($p<0.05$) at 5 and 7 days post-MI, respectively. Interestingly, we noted a selective increase of Ly6Chi monocytes (210% at 5 days, $p<0.05$ and 170% at 7 days, $p<0.05$) and Neutrophils (160% at 5 days, $p<0.05$ and 500% at 7 days, $p<0.01$) levels in D6-/- ischemic hearts. D6-/- mice were cardiac rupture-prone and displayed decreased post-MI survival ($p=0.02$ vs WT mice). 14 days after myocardial infarction, D6-/- mice showed features of adverse ventricular remodelling: end-diastolic and end-systolic endocardial volumes were increased by 40% and 50%, respectively ($p<0.01$), endocardial ejection fraction was reduced by 50% ($p<0.05$) and infarct size was increased by 30% ($p<0.05$). Leukocytes born D6 had no effect, as WT mice lethally irradiated and reconstituted with D6-/- bone marrow derived cells showed normal post-MI inflammation and remodelling. Altogether, these data demonstrate, for the first time, that the chemokine decoy receptor D6 limits MI-associated inflammation, and prevents adverse ventricular remodelling after MI.

0310

Inflammation in Atherosclerosis: Use of THP-1 monocytes for testing the NLRP3 inflammasome activity

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Atherosclerosis is an inflammatory disease in which circulating monocyte subsets infiltrate the intima and contribute to plaque development. They

differentiate into macrophages and produce inflammatory molecules such as IL-1β that is obtained from pro-IL-1β selectively cleaved by multiproteic complexes named inflammasomes. Our preliminary results established that NLRP3 inflammasome is expressed in plaques of LDLR-/- mice fed a High Fat Diet (HFD). The aim of this study is to set up a human cell pattern using human monocytes from ATCC to study the modulation of NLRP3 inflammasome activity by different stimuli.

Immunocytochemistry using 3 different antibodies performed on aortic valves cryosections obtained from mice fed a HFD for 10 weeks confirms the presence of NLRP3 marker into the plaques. We also evidenced labeled mononuclear circulating cells suspected to be activated monocytes. We cultured and differentiated human THP-1 monocytes using phorbol ester (TPA) at 20nM for 24 hours. Cells were then treated with LPS (10 and 100 ng/ml) for 6, 24 or 48 hours. Immunocytochemistry using the same antibodies demontrated an induction of NLRP3 expression at both LPS concentrations and at any times investigated. We measured the proinflammatory response of THP-1 cells cultured in the described differentiation conditions after 24 hours of LPS treatment through the release of IL-1β assessed by ELISA. Supernatants were harvested and cells collected and lysed in RIPA buffer. ELISA is processed on supernatants and cell lysates. Whatever the concentrations of LPS, THP-1 produced IL-1β, which quantities are 10-fold higher in the supernatants than in the cells.

NLRP3 inflammasome is expressed in THP-1 cells. Upon stimulation by LPS, NLRP3 is activated and able to process and secrete IL-1β. This assay will permit studying activators and inhibitors of NLRP3 and performing a screening of potential atherogenic and anti-atherogenic molecules.

0230

Hospital case fatality in HIV-infected patients with myocardial infarction. Analysis from a French nationwide hospital medical information database.

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Background: After more than two decades of AIDS epidemic, the spectrum of HIV-associated disease has considerably evolved from infectious disease with improved survival, to cardiovascular diseases and particularly coronary diseases due to chronic inflammation. As a consequence, myocardial infarctions are currently growing and few data are available on short-term prognosis in MI in HIV infected patients. The aim of this study was to examine the rate, characteristics and hospital case fatality of HIV-infected patients with MI.

Patients and methods: From the French nationwide hospital medical information database, data from all the consecutive patients hospitalized in the 1546 French hospital/clinics for myocardial infarction from 1st January 2005 to 31st December 2009 were analyzed. Data were extracted with codes of the International Classification of Diseases, tenth revision (ICD-10).

Results: Among the 677.305 patients included, the prevalence of HIV-infected patients was 0.2% (1347). HIV patients were younger, and more frequently male and smoker. By multivariate logistic regression analysis, age (OR=1.03), sex (OR=0.94), smoking (OR=0.70), hypertension (OR=0.67), diabetes (OR=0.91), dyslipidemia (OR=0.48), obesity (OR=0.86), atrial fibrillation (OR=0.85), PCI (OR=0.51), bypass (OR= 0.70) and STEMI (OR=4.22) are predicting factors for case fatality, while HIV infected patients were not associated with a worse prognosis (OR=1.03, p=0.802). However, in a subgroup of HIV infected patients matched for age, sex and STEMI with non HIV infected (matching ratio: 1:2), hospital case fatality showed a trend toward an increased risk in HIV group (3.12 vs 4.31%, p=0.053).

Conclusion: In our retrospective large nationwide study, the hospital case fatality of HIV-infected could be increased when compared with non HIV infected patients. Further stratified analysis are needed in order to better characterize the management and specific outcomes of HIV-infected patients.

0226

Acute myocardial infarction and breast cancer: data from the RICO survey

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Objective: Breast cancer is the most frequent cancer in French women population. Since few years its prognosis has been improved thanks to the screening and therapeutic progresses. Unfortunately, it is now well known that radiotherapy and chemotherapy can promote coronary events. However, to our knowledge, characteristics and management of acute myocardial infarction (AMI) in this population has not been determined yet that's why we decide to focus on.

Patients and methods: 73 patients with breast cancer history were identified among the 2087 women patients included in RICO (obseRvatoire des Infarctus de Côte d'Or) for AMI since 2001 to 2009. We matched up these 73 patients with 5 patients with an AMI, with respect to age, and gender in order to compare their clinical, angiographic and biological characteristics.

Results: Median age of these populations was 74 (65-80) years. No significant difference was found between these 2 populations considering cardiovascular risks. Type of MI (NSTEMI, STEMI) and its complications during the hospitalisation were not different between the 2 groups. Management and therapeutics begin during the acute phase seem not to be influenced by breast cancer history. However, we noticed that among biological parameters, there were 2 surprising statistical significant differences concerning CRP and CPK levels during AMI in favour of breast cancer population. Thus, CRP median level in breast cancer population was 1.00 mg/l (1.00–9.80) whereas it was equalled to 5.60 mg/l (2.80–13.60) in patients without history of breast cancer ($p<0.001$). Furthermore CPK median level was 310 UI/l (136–777) in breast cancer population, at the opposite the median level of this enzyme was 501 UI/l (198-1324) in patients without past of breast cancer (p=0.022).

Conclusions: These results suggest that MI size evaluated by CPK plasma level is smaller in patients with MI and breast cancer history than patients unharmed of breast cancer. Perhaps, hormonal therapy such as aromatase inhibitors and Tamoxifen take a part in the explanation of these results.

0343

Impact of age on clinical periodontal parameters in patients with acute myocardial infarction

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We aimed to evaluate the periodontal status in patients with acute myocardial infarction (AMI) and to determine whether there was a specific profile according to age.

Methods: In 197 consecutive AMI patients the number of teeth, endodontically treated teeth, periodontal screening index (PSI), clinical attachment level, and radiographic apical lesions were examined. Patients were classified according to tertiles of age.

Results: The study demonstrated that patients with AMI exhibited an unfavourable dental state of health. No relationship was found between C-reactive protein levels and periodontitis.

Conclusion: This work demonstrates specific profiles of dental status according to age. In younger patients, the dental status was poor, but no relationship with CRP was shown. Further studies are needed to include a more specific assessment of coronary lesions and their evolution in this context of poor dental health.

	Tertile 1	Tertile 2	Tertile 3	p
N	66	66	65	
Mean age, y	47.7±0.4	59.5±0.3	72.5±0.4	<0.001
Men	83%	89%	75%	0.104
CRP > 3 mg/L,	52%	41%	54%	0.205
Current smoker	72%	63%	47%	0.017
Periondontal status				
Presence of caries	50%	28%	45%	0.037
Presence of inflammation	64%	71%	67%	0.731
Teeth lost	8±8	10±8	15±8	0.019
Alteration of chewing	24%	34%	67%	<0.001
Bone Loss	55%	71%	86%	0.001

0344

Admission glycemia in acute myocardial infarction: incremental prognostic value for mortality over GRACE risk score and left ventricular ejection fraction. Data from the RICO survey

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Objective: High blood glucose (HBG) on admission is a major common metabolic disorder in patients with acute myocardial infarction (MI) and is associated with worse prognosis. However, only few data have examined its predictive performance over established risk score.

Patients and methods: From a French regional survey for acute MI, we analyzed the relationship between HBG, as defined by admission glycemia >11 mmol/L, and 1 year mortality in patients with acute MI. All multivariate Cox models were adjusted for the Global Registry of Acute Coronary Events (GRACE) risk score, which is a validated 9 variables prediction tool, and left ventricular ejection fraction as assessed by echocardiography <3 days after admission. The additional prognostic information of HBG was tested by comparing the -2log likelihood of the Cox models with vs without HBG (chi^2).

Results: In the study population (n=3358), both admission glycemia as a continuous variable and HBG were univariately associated with increased mortality (HR(95%CI: 1.06(1.05-1.07) and 2.67(2.17-3.29), respectively). The addition of either admission glycemia as a continuous variable (HR(95%CI): 1.04(1.01-1.06) or of HBG (HR(95%CI): 1.61(1.28-2.03)) significantly improved the risk prediction in the multivariate model (chi^2: $p<0.001$). However, in diabetic patients (n=756), HBG failed to independently predict mortality (HR(95%CI):1.17(0.80-1.71)). In contrast, in non diabetic patients (n=2592), HBG remained an independent predictor of death (HR(95%CI): 1.93(1.39-2.67)) and added incremental prognostic value in the model over the GRACE risk score and LVEF (chi^2: $p<0.001$).

Conclusion: High blood glucose on admission provides incremental prognostic information over established risk score and LVEF, in particular in non diabetic patients. Admission glycemia is not an independent predictive marker in diabetic patients.